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C2C CAA CKF CKJ CKP CKR C1175 C1178 C1200 C1230 C1530 C1532 C1535 C213 C215 C22Y C220 C221 C225 C247 C25Y C250 C251 C271 C280 C281 C282 C30Y C31Y C311 C313 C32Y C322 C332 C337 C34Y C342 C36Y C360 C361 C364 C579 C604 C62X C620 C623 C624 C644 C660 C662 C670 C672 C697 C80Y C802 U1S S1321 S2416

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Chem. Abs. 111:153648 & JP01113369 A2.(MITSUBISHI PETROCHEMICAL CO.) Chem. Abs. 120:217217 & Khim.-Farm. Zh. (1993), 27(7), 34-5

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UK CL (Edition P) C2C CKH CKJ CKP CKT
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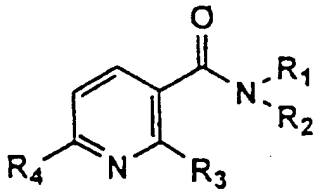
(54) Abstract Title
Nicotinic acid amide derivatives

(57) A nicotinic acid amide derivative of formula (I), pharmacologically acceptable salts and pharmaceutical compositions thereof:



(I)

wherein R_1 is H or lower alkyl; R_2 is optionally substituted pyridinyl, pyridinemethyl, N-benzylpiperidinyl, isoquinolyl or benzyl; R_3 is optionally substituted cyclopentyl, cyclohexyl, cyclooctyl, norbornyl, adamantyl, piperidyl, pyridyl, isoquinolyl or azabicyclooctyl wherein the ring system is attached to the nicotinamide ring by means of an oxygen or NH group and R_4 is H or lower alkoxy. The compounds are useful for the prevention and treatment of stroke, brain edema after stroke and a variety of allergic and inflammatory diseases, e.g. asthma, chronic bronchitis, shock, rheumatoid arthritis, reperfusion injury, encephalomyelitis and multiple sclerosis. The compounds are prepared from intermediates wherein R_3 is chlorine which in turn are prepared from reaction of the corresponding acid with HNR_1R_2.

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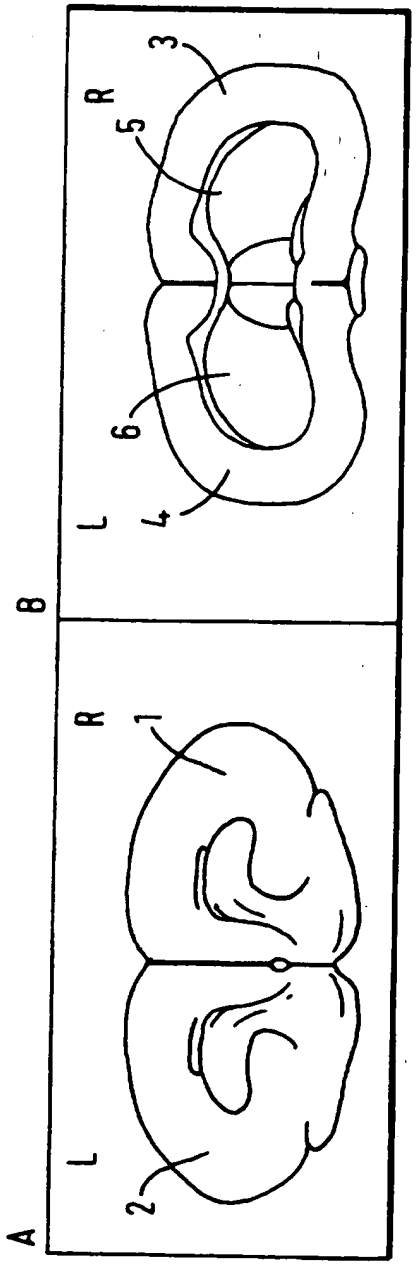


FIG. 1

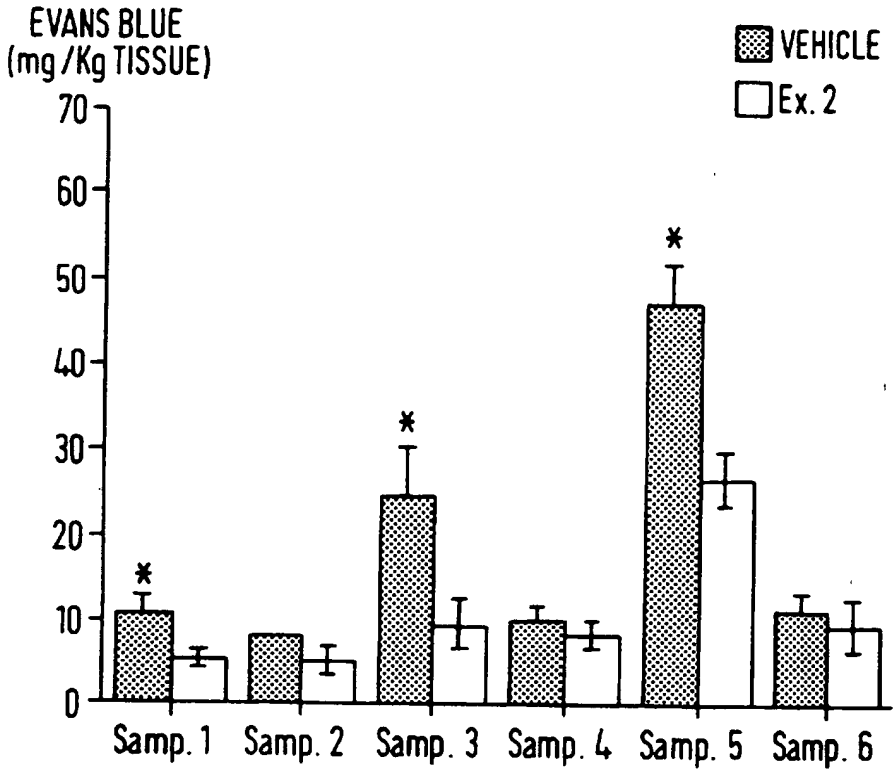
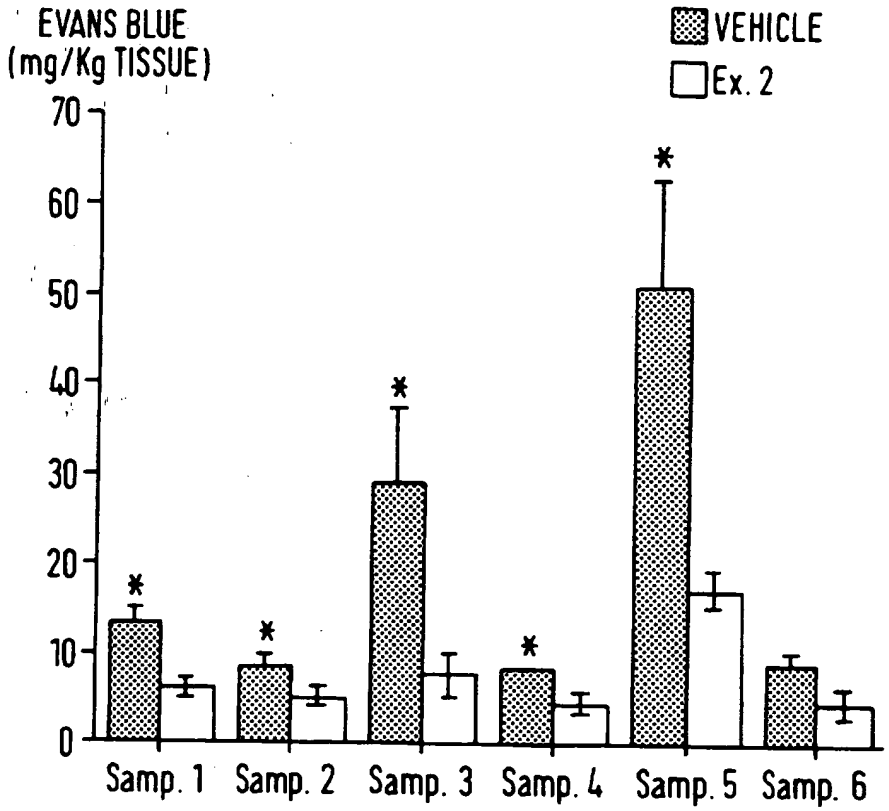

EVANS BLUE
(mg/Kg TISSUE)
VEHICLE
Ex. 2
70
60
50
40
30
20
10
0
*
*
*
Samp. 1
Samp. 2
Samp. 3
Samp. 4
Samp. 5
Samp. 6

FIG. 2


EVANS BLUE
(mg/Kg TISSUE)
VEHICLE
Ex. 2
70
60
50
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Samp. 1
Samp. 2
Samp. 3
Samp. 4
Samp. 5
Samp. 6

FIG. 3

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NICOTINAMIDE DERIVATIVES AND THEIR USE AS MEDICAMENTS

The present invention relates to a novel nicotinamide derivative which is useful for the prevention, treatment or amelioration of stroke, brain edema after stroke and a variety of allergic and inflammatory diseases such as asthma, chronic bronchitis, shock, rheumatoid arthritis, reperfusion injury, encephalomyelitis and multiple sclerosis.

Recently, patients suffering from allergic or inflammatory diseases are increasing markedly. These diseases severe symptoms and are intractable and can be recurrent. There are no therapeutic drug having high effectiveness and safety. Therefore, a therapeutic drug having high efficacy and safety in the clinical field has been strongly desired.

Cyclic 3',5'-adenosine monophosphate (hereinafter abbreviated as cAMP) is a well known second messenger that mediates the functional responses of cells to hormones, autocoids, neurotransmitters and drugs, Sutherland et al Pharmacol Rev 12 265 (1996). The cellular levels of cAMP are regulated by mechanisms which control its synthesis and breakdown. The breakdown of cAMP is controlled by a family of phosphodiesterases (hereinafter abbreviated to PDE) (Beavo et al TiPS 11, 1150, 1990). It has been shown that PDE IV plays a main role to regulate cAMP concentrations in airway smooth muscle and inflammatory cells, Dent et al British J Pharmacology 90, 163, (1990). and inhibition of PDE IV can lead to prevent inflammatory mediator release, Verghese et al J Mol Cell Cardiol 12 (Suppl II), S61 (1989). Thus, compounds that inhibit PDE IV would be useful for the treatment of inflammatory disease.

In the meantime, brain capillary endothelial cells (hereinafter abbreviated as ECs) form the blood-brain barrier (hereinafter abbreviated as BBB), which is usually spoken of as having an essential role in maintaining the normal extracellular environment of the central nervous system (hereinafter abbreviated as CNS). The BBB is a real molecular barrier, permitting only small hydrophobic molecules, a limited set of specifically transported nutrients (glucose and certain amino acids), and a restricted number of specifically transcytosed macromolecules, such as transferrin, entry into the brain. Two separate properties of brain capillary ECs account for the limited molecular transport: their low rates of fluid-phase endocytosis (and correspondingly low rates of transcellular flux) and their coupling by high electrical resistance tight junctions (which severely limit paracellular flux).

For some time, the BBB has been known to be clinically important. When tight junctions are grossly disrupted, as sometimes happens following stroke, the resulting entry of proteins and ions into the brain leads to edema because of the associated influx of water. Even an apparently normal BBB can be breached, as occurs with the entry of certain types of lymphocytes and metastatic cells into the brain; such

conditions are associated with serious disease (multiple sclerosis and metastatic brain tumour. for example).

In the case of cultured brain ECs, addition of a membrane permeant cyclic AMP derivative has been shown to decrease tight junction permeability and to produce a reorganization of the actin cytoskeleton, reducing the number of stress fibres, thereby yielding a well defined cortical actin belt. It follows that inhibition of cyclic AMP phosphodiesterase (hereinafter abbreviated as PDE) activity could produce a similar effect. This is indeed the case as inhibitors of PDE IV, such as rolipram, do decrease tight junction permeability.

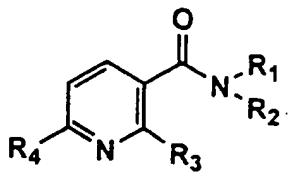
In the case of stroke, PDE IV inhibitors would be useful agents to decrease the vasogenic edema that severely complicates this condition. By reversing the increased permeability of the tight junctions of the brain endothelial cells, the entry of ions and protein into the brain would be prevented and the CNS environment would return to normal. Vasogenic edema would be prevented.

In multiple sclerosis, activated T cells bind to the brain endothelium and transmigrate to enter the CNS. If CNS antigen is encountered, the cells remain to trigger an inflammatory cascade, ultimately resulting in demyelination. Transmigration of activated T cells across brain endothelial cells can be inhibited in vitro by PDE IV inhibitors. The mechanism of inhibition may be due to blockade of T cell-initiated, endothelial signalling processes that are necessary for transmigration. It follows that PDE IV inhibitors could be agents to block activated T cell entry into the brain in multiple sclerosis, thereby providing a potential therapy.

In those diseases, cyclic AMP Phosphodiesterase (hereinafter, abbreviated as PDE) plays a important role. For example, it is disclosed that Rolipram, a selective type IV PDE inhibitor, is a potential anti multiple sclerosis drug [Nature Medicine, 1(3),244-248,1995.]. Furthermore, it is disclosed also that PDE inhibitors can prevent experimental allergic encephalomyelitis [Proc.Natl,Acad,Sci,USA,92(4),3601-3605,1995.]. Since Rolipram is an antidepressant, adverse reactions such as sleepiness, lowering of concentration or reflex movement ability are unavoidable.

Regarding the foregoing problems, the present inventors have proceeded with extensive research. As a result, it has been found that a novel nicotinamide derivative represented by the formula (I) has excellent efficacy and safety.

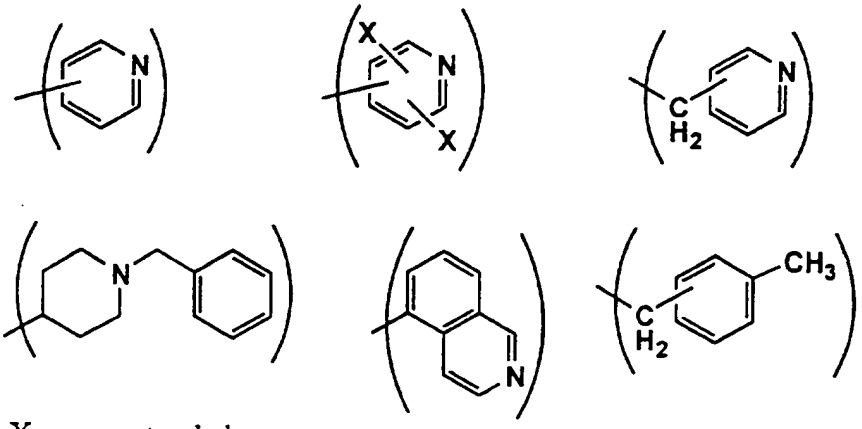
According to a first aspect of the present invention there is provided a compound of general formula (I):



(I)

wherein R^1 represents a hydrogen atom or a lower alkyl group;

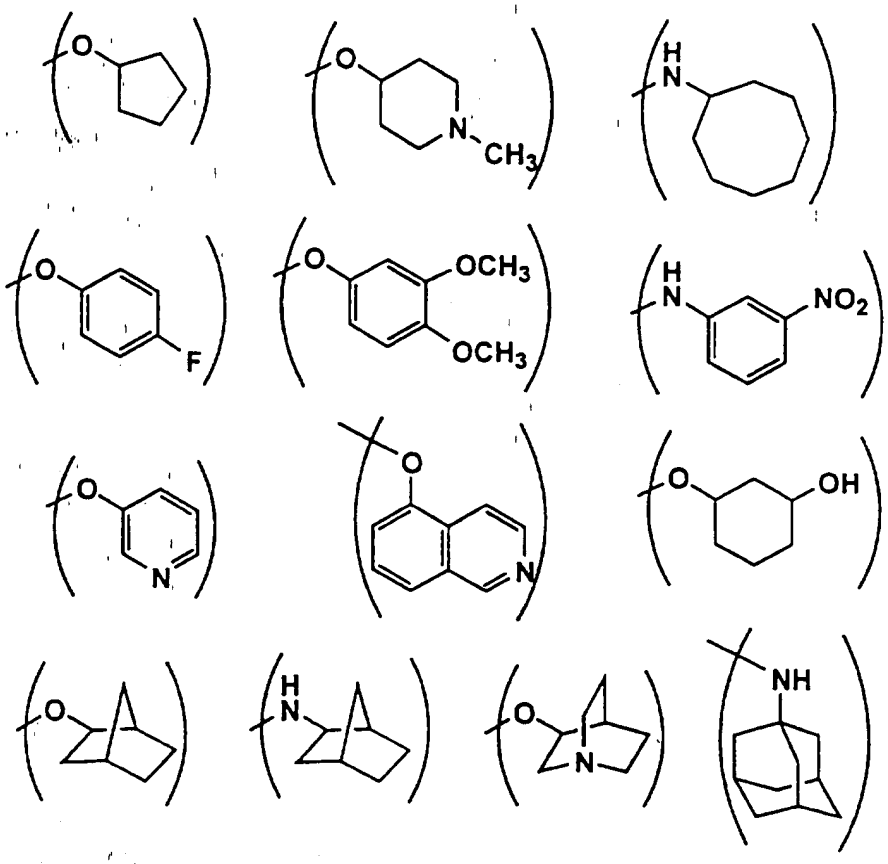
R^2 represents a group selected from the following group:



wherein X represents a halogen atom;

or R^1 and R^2 can form a 4-methylpiperazinyl group together which may be substituted;

R^3 represents a group selected from the following group;



R^4 represents a hydrogen atom or a lower alkyl group.

The present invention offers a potential anti allergic and inflammatory drug, especially for asthma, chronic bronchitis, shock, rheumatoid arthritis, reperfusion injury, encephalomyelitis and multiple sclerosis.

With respect to the above definition of the above formulas, particular examples of the halogen atom include chlorine atom, fluorine atom, bromine atom and iodine atom, among which chlorine atom is preferable. Particular examples of the lower alkyl group include alkyl groups having 1 to 6 carbon atoms, such as methyl group, ethyl group, n-propyl group, i-propyl group, n-butyl group, i-butyl group, t-butyl group, pentyl group and hexyl group.

More specific examples of the nicotinamide derivative represented by the above formula (I) according to the present invention include the following compounds, though the nicotinamide derivative is not limited to them;
(1) N-(4-pyridyl)-2-cyclopentyloxynicotinicamide,
(2) N-(4-pyridyl)-2-exonorbornyloxynicotinic amide,
(3) N-(4-pyridyl)-2-(4-fluorophenyloxy) nicotinic amide,
(4) N-(4-pyridyl)-2-(3-hydroxycyclohexyloxy) nicotinic amide,

(5) N-(4-pyridyl)-2-(2-exonorbornylamino) nicotinic amide,
(6) N-(4-pyridyl)-2-cyclooctylaminonicotinic amide,
(7) N-(4-pyridyl)-2-adamantylaminonicotinic amide,
(8) N-(4-pyridyl)-2-exonorbornyloxy-6-methyl nicotinic amide,
(9) N-(3-pyridyl)-2-exonorbornyloxynicotinic amide,
(10) N-(3,5-dichloro-4-pyridyl)-2-(2-exonorbornyloxy) nicotinic amide,
(11) N-(5-isoquinolynyl)-2-(2-exonorbornyloxy) nicotinic amide,
(12) N-(4-picolyl)-2-(4-fluorophenyloxy) nicotinic amide,
(13) N-(4-picolyl)-2-(3-nitrophenylamino) nicotinic amide,
(14) N-(3-picolyl)-2-(2-Exonorbornyloxy) nicotinic amide,
(15) N-(3-picolyl)-2-(3,4-dimethoxyphenyloxy) nicotinic amide and;
(16) N-(1-Benzyl-4-piperidyl)-2-(2-exonorbornyloxy) nicotinic amide.

The present invention provides a method for treating a variety of allergic and inflammatory diseases such as asthma, chronic bronchitis, shock, rheumatoid arthritis, reperfusion injury, encephalomyelitis and multiple sclerosis, etc. accompanied by PDE IV activity by administering to a human patient a pharmacologically effective amount of a compound according to general formula (I) above for inhibiting the PDE IV activity. In other words, there is provided the use of a compound or a pharmacologically acceptable salt thereof according to the present invention for the making of a medicament for treating or ameliorating a disease against which phosphodiesterase antagonsim is efficacious.

The invention further provides a therapeutic composition which comprises a pharmacologically effective amount of a compound according to general formula (I) above and a pharmacologically acceptable carrier.

Specifically, the compounds of general formula (I) of the present invention may be effective for treatment, prevention, remission, improvement, etc. of a variety of allergic and inflammatory diseases such as asthma, chronic bronchitis, shock, rheumatoid arthritis, reperfusion injury, encephalomyelitis and multiple sclerosis.

Where a compound of general formula (I) of the present invention is used as a pharmaceutical agent in the treatment or amelioration for these diseases, it may be orally or parenterally administered. In general, it is parenterally administered in the form of injections, such as intravenous, subcutaneous, and intramuscular injections, suppositories, or sublingual tablets. The dose will remarkably vary depending upon the symptom; age, sex, weight, and sensitivity of patients; method of administration; time and intervals of administration and properties, dispensing, and kind of pharmaceutical preparations; kind of effective ingredients, etc., so that there is no particular limitation with respect to the dose. Normally the compound may be administered in a dose of about 0.1 to 1000 mg, preferably 0.5 to 500 mg, more preferably 1 to 100 mg, per day per adult, ordinarily in one to four portions.

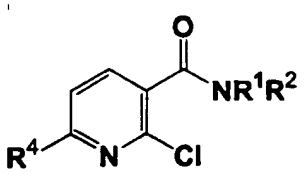
In preparing injections, the effective ingredient may be blended, if necessary, with a pH modifier, a buffer, a suspending agent, a solubilizing agent, a stabilizer, a tonicity agent, a preservative, etc., followed by preparation of an intravenous, subcutaneous, or intramuscular injection according to an ordinary method. In this case, if necessary, these preparations may be lyophilized according to an ordinary method.

Examples of the suspending agents include methylcellulose, Polysorbate 80, hydroxyethylcellulose, acacia, powdered tragacanth, sodium carboxymethylcellulose, and polyoxyethylene sorbitan monolaurate.

Examples of the solubility agent include polyoxyethylene hydrogenated castor oil, Polysorbate 80, nicotinamide, polyoxyethylene sorbitan monolaurate, Macrogol, and an ethyl ester of castor oil fatty acid.

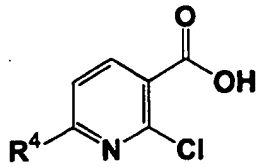
Examples of the stabilizer include sodium sulfite, sodium metasulfite and ether, and examples of the preservative include methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate, sorbic acid, phenol, cresol, and chlorocresol.

According to a further aspect of the present invention there is provided a process for the preparation of compound of general formula (II),



(II)

comprising derivatising an optionally protected compound of general formula (III),



(III)

and optionally thereafter converting the compound of general formula (II) so formed into another compound of general formula (II), in which R^1 to R^4 have the same meaning as defined in accordance with the first aspect of the present invention.

In this process, the derivativisation of the compound of general formula (III) to form a compound of general formula (II) may be carried out by treatment with:

(a) a chlorinating agent
(b) a mixed acid anhydride forming agent or,
(c) 1,3-dicyclohexylcarbodiimide (DCC),

and a primary or secondary amine of general formula (IV),

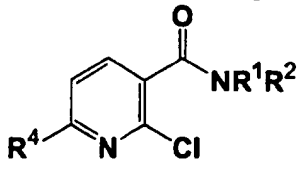
HNR^1R^2 (IV)

wherein R^1 and R^2 have the same meaning as defined in accordance with the first aspect of the present invention.

The chlorinating agent may conveniently be thionyl chloride, sulfuryl chloride, oxalyl chloride, phosphorus pentachloride, phosphorous trichloride or phosphorous oxychloride. The mixed acid anhydride forming agent may be methyl chloroformate or ethyl chloroformate.

The compound of general formula (II) prepared by a process in accordance with this aspect of the present invention may be further treated with an alcohol or an amine to give a compound of general formula (I).

The present invention also extends to a compound of general formula (II), in which R^1 to R^4 have the same meaning as defined in claim 1,
The present invention also extends to a compound of general formula (II), in which R^1 to R^4 have the same meaning as defined in claim 1,



(II)

with the proviso that N-(4-pyridyl)-2-chloronicotinic amide, N-(3-pyridyl)-2-chloronicotinic amide and N-(2-pyridyl)-2-chloronicotinic amide are excluded.

More specific examples of such compounds include,

(1) N-(4-pyridyl)-2-chloro-6-methyl nicotinic amide,
(2) N-(3,5-dichloro-4-pyridyl)-2-chloronicotinic amide,
(3) N-(5-isoquinolyl)-2-chloronicotinic amide,
(4) N-(4-picolyl)-2-chloronicotinic amide,
(5) N-(3-picolyl)-2-chloronicotinic amide and;
(6) N-(N-benzylpiperidin-4-yl)-2-chloronicotinic amide.

Preferred features of the second and subsequent features are as for the first aspect *mutatis mutandis*.

The invention will now be described by way of example with reference to the accompanying Examples which are provided for the purposes of illustration and are not to be construed as being limiting on the present invention. Reference is made in the Examples to a number of Figures in which:

FIGURE 1 shows coronal sections of rat brain illustrating division of right (R) and left (L) hemispheres into six regions for measurement of tissue Evans Blue content.

A - level anterior to infarct (bregma + 2.7mm);
B - level of infarct (bregma - 0.3mm).

FIGURE 2 shows the effect of the compound of example 2 (5 hour infusion) on BBB disruption.

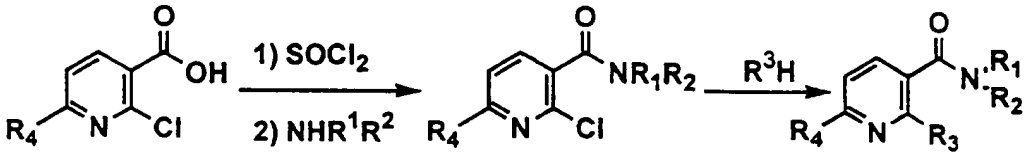
Evans Blue was extracted from 6 areas of brain 5 hours after MCAo as described in the test. The compound was administered after onset of occlusion. Data are expressed as mean ± SE. The compound (n=5) vs. vehicle (n=5) *p<0.05 (Student T-test).

FIGURE 3 shows the effect of the compound of example 2 (48 hour infusion) on BBB disruption.

Evan Blue was extracted from 6 areas of the brain 48 hours after MCAo as described in the test. The compound was administered after onset of occlusion. Data are expressed as mean ± SE. The compound (n=6) vs vehicle (n=7) * p<0.05 (Student T-test).

Preparative Examples

(1) Synthetic route



"intemediate" (I)

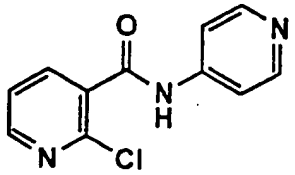
(wherein R^1 to R^4 have the same meaning as defined above.)
2-Chloronicotinic acid or its 6-substituted derivative was treated with;
1) chlorinating agent (e.g., thionyl chloride, sulfuryl chloride, oxalyl chloride, phosphorous pentachloride, phosphorous trichloride, phosphorous oxychloride) or
2) mixed acid anhydride forming agent (e.g., methyl chloroformate, ethyl choloroformate) or

3) 1,3-dicyclohexylcarobodiimide (DCC);
and primary or secondary amine to afford intermediate amide compounds, and this was treated with alcohol or amine to give the nicotinamide derivative (I).

(2) Synthesis of intermediates

Preparative Example 1 (intermediate 1)

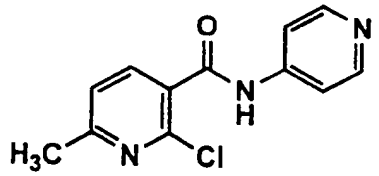
N-(4-pyridyl)-2-chloronicotinic amide



The mixture of 2-chloronicotinic acid (15g,0.095mol) and thionyl chloride(150ml) was heated at 60°C for 8 hours followed by evaporation of excess thionyl chloride to give crude acid chloride. This was washed with ether and dried and then dissolved in 150ml of dichloromethane. To the solution of 4-aminopyridine (10g,0.1mol) and triethylamine (20ml) in 200ml of dichloromethane was added the solution of above acid chloride in dichloromethane at 0°C for 30 minutes and stirred for 10 hours at room temperature (hereinafter abbreviated as RT).
The reaction mixture was poured onto water and extracted with dichloromethane twice, combined organic layer was washed with brine and dried over $MgSO_4$. The dichloromethane was evaporated to dryness to afford 20g of the titled compound.
^{1}H-NMR($CDCl_3$); δ(ppm) 8.82 (brs,1H), 8.52(m,3H), 8.14(d-d,1H,J=7Hz,2Hz), 7.58 (d,2H,J=8Hz).

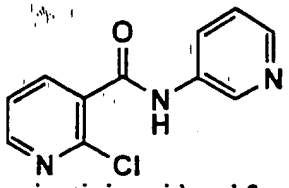
Preparative Example 2 (intermediate 2)

N-(4-pyridyl)-2-chloro-6-methyl nicotinic amide



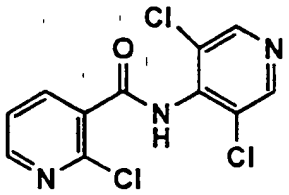
2-Chloro-6-methylnicotinic acid and 4-aminopyridine were reacted according to the synthesis of (1) to give the titled compound.
^{1}H-NMR($CDCl_3$); δ(ppm) 8.58(d,2H,J=7Hz), 8.52(brs,1H), 8.14(d,2H,J=7Hz), 7.60(d,2H,J=7Hz), 7.29(d,2H,J=7Hz), 2.60(s,3H).

Preparative Example 3 (intermediate 3)
N-(3-pyridyl)-2-chloronicotinic amide



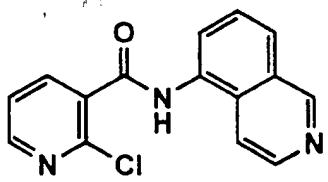
2-Chloronicotinic acid and 3-aminopyridine were reacted according to the synthesis of (1) to give the titled compound.

Preparative Example 4 (intermediate 4)
N-(3,5-dichloro-4-pyridyl)-2-chloronicotinic amide



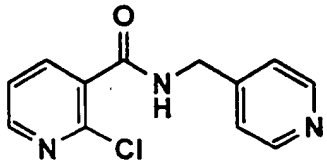
2-Chloronicotinic acid and 3,5-dichloro-4-aminopyridine were reacted according to the synthesis of (1) to give the titled compound.

Preparative Example 5 (intermediate 5)
N-(5-isoquinolyl)-2-chloronicotinic amide



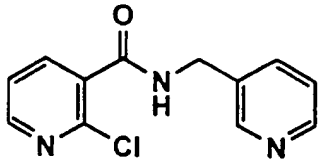
2-Chloronicotinic acid and 5-aminoisoquinoline were reacted according to the synthesis of (1) to give the titled compound.

Preparative Example 6 (intermediate 6)
N-(4-picolyl)-2-chloronicotinic amide



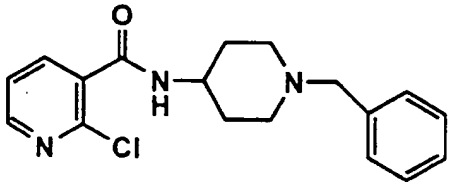
2-Chloronicotinic acid and 4-picolylamine were reacted according to the synthesis of (1) to give the titled compound.

Preparative Example 7 (intermediate 7)
N-(3-picolyl)-2-chloronicotinic amide



2-Chloronicotinic acid and 3-picolylamine were reacted according to the synthesis of (1) to give the titled compound.

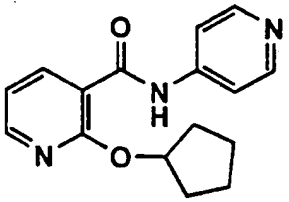
Preparative Example 8 (intermediate 8)
N-(N-benzylpiperidin-4-yl)-2-chloronicotinic amide



2-Chloronicotinic acid and 1-benzyl-4-aminopiperizine were reacted according to the synthesis of (1) to give the titled compound.

EXAMPLES

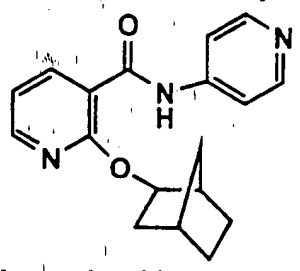
Example 1
N-(4-pyridyl)-2-cyclopentyloxynicotinicamide



To the suspension of 50% of sodium hydride (4.3g,0,09mol) in dimethylformamide was added cyclopentanol (7.74g,0,09mol) at RT and stirred for 1 hour and then the intermediate (1) was added and reacted at 110-120°C for 4 hours. The reaction mixture was poured onto ice-water and extracted with ethyl acetate(twice) and the organic layer was washed with water, brine and dried over $MgSO_4$. The organic phase was evaporated to dryness and the residue was purified on 200g of silica gel column chromatography (2% ethanol-dichloromethane) to afford 9.0g of the titled compound. ^{1}H-NMR(D_2O) ; δ(ppm) 8.50(d,2H,J=7Hz), 8.20(m,2H), 8.03(d,2H,J=7Hz), 7.10(d-d,1H,J=7Hz,7Hz), 5.40(m,1H), 1.5-2.0 (m,8H).

Example 2

N-(4-pyridyl)-2-exonorbornyloxynicotinic amide

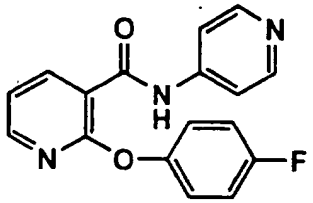


Exonorborneol and intermediate (1) were reacted according to the procedure of example (1) to afford the titled compound.

^{1}H-NMR($CDCl_3$) ; δ(ppm) 10.30(brs,1H), 8.52(m,3H), 8.30(d-d,1H,J=7Hz,1Hz), 7.58(d,2H,J=7Hz), 7.06(d-d,1H,J=7Hz,7Hz), 5.18(d,1H,J=5Hz), 2.60(m,1H), 2.42(m,1H), 1.1-2.1(m,8H),

Example 3

N-(4-pyridyl)-2-(4-fluorophenyloxy) nicotinic amide

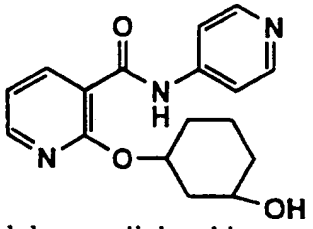


4-Fluorophenol and intermediate (1) were reacted according to the procedure of example (1) to afford the titled compound.

^{1}H-NMR (HCl salt in D_2O) ; δ(ppm) 8.57(d,2H,J=7Hz), 8.25(d-d,1H,J= 7Hz,1Hz), 8.10(m,3H), 7.30(d-d,1H,J=7Hz,7Hz), 7.15(d,4H,J=7Hz).

Example 4

N-(4-pyridyl)-2-(3-hydroxycyclohexyloxy) nicotinic amide

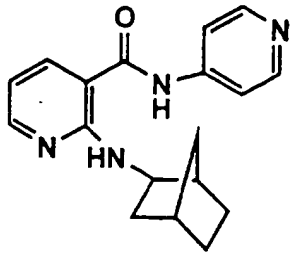


1,3-Cyclohexanediol and intermediate(1) were reacted according to the procedure of example (1) to afford the titled compound.

^{1}H-NMR($CDCl_3$) ; δ(ppm) 10.35(brs,1H), 8.56(m,3H), 8.30(d-d,1H, J=7Hz,1Hz), 7.68(d,2H,J=7Hz), 7.12(d-d,1H,J=7Hz,7Hz), 5.48(m,1H), 4.00(brs,1H), 1.4-2.4(m,8H).

Example 5

N-(4-pyridyl)-2-(2-exonorbornylamino) nicotinic amide

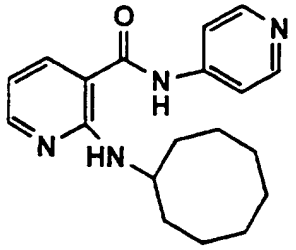


To the solution of exo-2-norbornyl amine (710mg,6.4mM) and intermediate (1) (1.5g,6.4mM) in 15ml of dimethylformamide was added copper(II) acetate (58mg,0.32mM) and n-ethylmorphorine(0.8ml) and heated to 110°C for 15 hours. The reaction mixture was poured onto ice-water and extracted with ethyl acetate(three times) and the organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified on silica gel column chromatography (3% ethanol-dichloromethane) to give 1.1g of the titled compound.

^{1}H-NMR ($CDCl_3$); δ(ppm) 8.30(d,2H,J=7Hz), 7.94(brs,2H), 7.70(d-d,1H,J=7Hz,1Hz), 7.50(brs,2H), 6.51(d-d,1H,J=7Hz,7Hz), 3.80(m,1H), 2.30(brs,2H), 1.1-2.0(m,8H).

Example 6

N-(4-pyridyl)-2-cyclooctylaminonicotinic amide

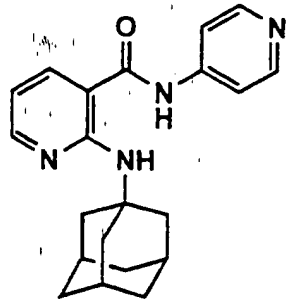


Cyclooctylamine and intermediate (1) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR($CDCl_3$); δ(ppm) 8.50(d,2H,J=7Hz), 8.28(m,1H), 8.12(brs,1H), 8.08(d,J=10Hz), 7.70(d,1H,J=7Hz), 7.52(d,2H,J=7Hz), 6.50(d-d,1H,J=7Hz,7Hz), 4.30 (m,1H), 1.4-2.1(m,14H).

Example 7

N-(4-pyridyl)-2-adamantylaminonicotinic amide

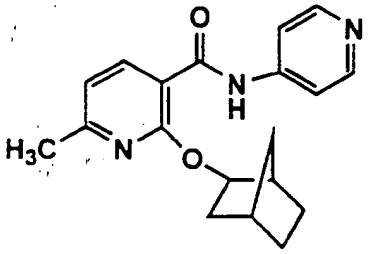


Adamantylamine and intermediate (1) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR($CDCl_3$); δ(ppm) 8.50(d,2H,J=7Hz), 8.22(m,1H), 8.13(brs,1H), 7.90(brs,1H), 7.72(d-d,1H,J=7Hz,1Hz), 7.53(d,2H,J=7Hz), 6.48(d-d,1H,J=7Hz,7Hz), 1.6-2.3(m,15H).

Example 8

N-(4-pyridyl)-2-exonorbornyloxy-6-methyl nicotinic amide

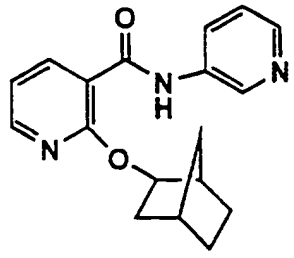


Exonorborneol and intermediate (1) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR($CDCl_3$); δ(ppm) 10.33(brs,1H), 8.55(d,2H,J=7Hz), 8.42(d,1H,J=7Hz), 7.59(d,2H,J=7Hz), 6.92(d,1H,J=7Hz), 5.21(m,1H), 2.50(s,3H), 1.2-2.6(m,10H).

Example 9

N-(3-pyridyl)-2-exonorbornyloxynicotinic amide

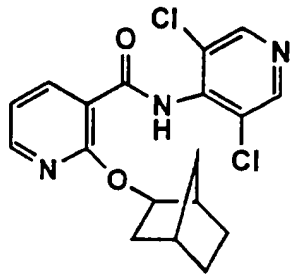


Exonorborneol and intermediate (3) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR(HCl salt in D_2O); δ(ppm) 9.29(s,1H), 8.43(d,1H,J=7Hz), 8.28(m,1H), 8.12(m,2H), 7.88(d-d,1H,J=7Hz,7Hz), 7.01(d-d,1H,J=7Hz,7Hz), 4.76(m,1H), 2.38(m,1H), 2.20(m,1H), 1.0-1.8(m,8H).

Example 10

N-(3,5-dichloro-4-pyridyl)-2-(2-exonorbornyloxy) nicotinic amide

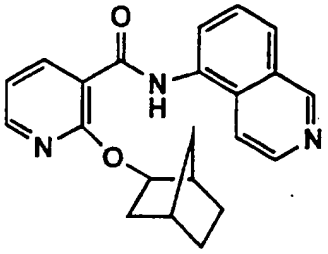


2-Exonorborneol and intermediate (4) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR ($CDCl_3$); δ(ppm) 9.92(brs,1H), 8.60(m,1H), 8.57(s,2H), 8.36(m,1H), 7.08(d-d,1H,J=7Hz,1Hz), 5.20(m,1H), 1.0-2.6(m,10H).

Example 11

N-(5-isoquinolynyl)-2-(2-exonorbornyloxy) nicotinic amide

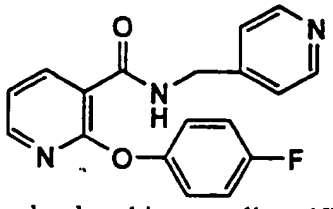


2-Exonorborneol and intermediate (5) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR(D_6-DMSO); δ(ppm) 10.65(s,1H), 9.95(s,1H), 8.78(d,1H,J=7Hz), 8.51(d,1H,J=7Hz), 8.40(m,3H), 8.10(m,2H), 7.18(d-d,1H,J=7Hz,7Hz), 5.02(d,1H,J=5Hz), 1.1-2.5(m,10H).

Example 12

N-(4-picolyl)-2-(4-fluorophenyloxy) nicotinic amide

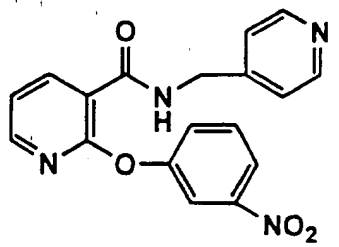


4-Fluoropheol and intermediate (6) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR ($CDCl_3$); δ(ppm) 8.55(m,2H), 7.43(d,1H,J=7Hz), 7.2-7.4(m,3H), 7.04(m,2H), 6.80(t,2H,J=8Hz), 6.20(t, H,J=7Hz), 5.04(s,2H).

Example 13

N-(4-picolyl)-2-(3-nitrophenylamino) nicotinic amide

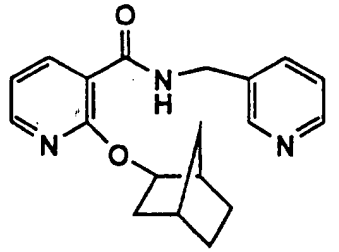


3-Nitroaniline and intermediate (6) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR($CDCl_3$); δ(ppm) 9.08(t,1H,J=5Hz), 8.87(m,1H), 8.55(d,2H, J=7Hz), 8.39(m,1H), 8.16(d-d,1H,J=7Hz,1Hz), 7.88(d,1H,J=7Hz), 7.78(d,1H,J=7Hz), 7.40(t,1H,J=7Hz), 7.28(d,2H,J=7Hz), 6.86(d-d,1H,J-7Hz,1Hz), 4.58(d,2H,J=5Hz).

Example 14

N-(3-picolyl)-2-(2-Exonorbornyloxy) nicotinic amide

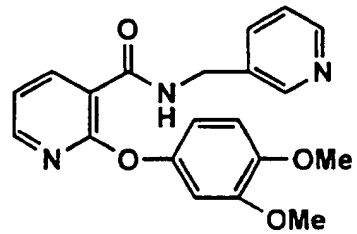


2-Exonorborneol and intermediate (6) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR($CDCl_3$); δ(ppm) 8.62(brs,1H), 8.55(m,1H), 8.50(d-d,1H,J=7Hz,1Hz), 8.40(m,1H), 8.25(m,1H), 7.70(d-d,1H,J=7Hz,1Hz), 7.28(d-d,1H,J=7Hz,7Hz), 7.03(d-d,1H,J=7Hz,7Hz), 5.03(m,1H), 4,65(d,2H,J=5Hz), 3.78(m,1H), 1.0-2.4(m,10H).

Example 15

N-(3-picolyl)-2-(3,4-dimethoxyphenyloxy) nicotinic amide

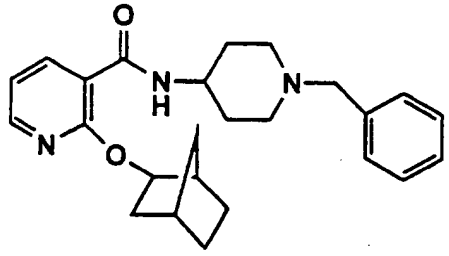


3,4-Dimethoxyphenol and intermediate (6) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR (HCl salt in D_2O); δ(ppm) 8.70(s,1H), 8.60(d,1H,J=7Hz), 8.45(d,1H,J=8Hz), 8,19 (d-d,1H, J=7Hz,1Hz), 8.12(m,1H), 7.90(d-d,1H,J=7Hz,7Hz), 7,22(d-d,1H,J=7Hz,7Hz), 6.96(d,1H,J=7Hz), 6,76(s,1H), 6.62(m,1H), 4.68(s,2H), 3.76(s,3H), 3.68(s,3H).

Example 16

N-(1-Benzyl-4-piperidyl)-2-(2-exonorbornyloxy) nicotinic amide



2-Exonorborneol and intermediate (8) was reacted according to the procedure of example (5) to afford the titled compound.

^{1}H-NMR ($CDCl_3$); δ(ppm) 8.46(d-d,1H,J=7Hz,1Hz), 8.20(m,1H), 8.08 (d,1H,J-7Hz), 7.2-7.3 (m,5H), 6.98(d-d,1H,J=7Hz,7Hz), 5.08(m,1H), 4.00(m,1H), 3.53(s,2H), 2.7-2.9(m,2H), 1.1-2.5(m,16H).

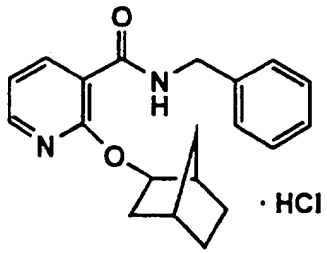
PHARMACOLOGICAL EXPERIMENTS

(1) *In vitro* study: Effects on transcellular resistance (TER) of pig brain endothelial cell cultures

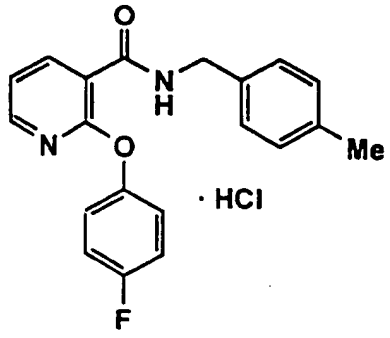
1) Materials

The compound of example 2 was used as a representative of the present invention. Rolipram was used as a positive control, and two compounds disclosed in the example 4 and 8 of US-4,861,891 (EP-357,316), close to the present invention structure, were used as controls.

Control 1



Control 2



2) Method

Microvessel isolation and pig brain endothelial cell (hereinafter abbreviated as PBEC) culture;

Essentially as described in Rubin et al. 1991[1] for bovine brain endothelial cells. Pig cortex is homogenized and microvessel fragments collected by filtration. Vessel fragments are cultured for 6 days, passaged and PBEC plated on collagen-treated polycarbonate Transwells. After 4 days the medium is changed to 50% astrocyte-conditioned medium (ACM), 50% defined medium [(hereinafter abbreviated as DMEM) with 10μg/ml transferrin, 100μM putrescine and 30nM sodium selenite], using a total volume of 1 ml, 250μl in upper chamber, 750μl in lower chamber. Transcellular electrical resistance (hereinafter abbreviated as TER) across the PBEC monolayer is determined using an EVOM resistance system (World Precision Instruments, Hertfordshire, UK). Resistance is corrected for resistance across an empty filter and expressed as Ohms × cm^2 (Ωcm^2). Electrical resistance across the monolayer exceeds 100 Ohms × cm^2 (Ωcm^2) before addition of test compounds.

Astrocyte-conditioned medium

Cultures of astrocytes that are over 95% pure are prepared from 1 day old Sprague-Dawley rat cortex, essentially as described by Lillien and coworkers (1988)[2]. Conditioned medium is collected every 2 days from confluent cultures.

Treatment with phosphodiesterase inhibitors

Five days after plating on Transwells untreated TER measurements are taken. Water insoluble test compounds are added at 1000 times concentration to the lower chamber in 1μl DMSO. Water soluble compounds are used at 100 times concentration in water with 2.5μl added to the upper and 7.5μl added to the lower chamber. TER measurements are made 1.5 hours, 2.5 hours and 24 hours after addition of drugs. Each concentration of each drug is tested on triplicate Transwells and each set of experiments includes a triplicate set of transwells treated with 10μM rolipram and another treated with DMSO (and water if appropriate) alone as controls. Percent change in TER from the starting reading for each Transwell is calculated and results expressed as mean ± standard deviation.

[1]; Rubin, L. L., Hall, D. E., Porter, S., Barbu, K., Cannon, C., Horner, H. C., Jantapour, M., Liaw, C. W., Manning, K., Morales, J., Tanner, L. I., Tomaselli, K. J. and Bard F. (1991). A cell culture model of the blood-brain barrier. J. Cell Biol. 115, 1725-35.

2) ; Lillien, L. E., Sendtner, M., Rohrer, H., Hughes, S. M., and Raff, M. C. (1988). Type-2 astrocyte development in rat brain cultures is initiated by a CNTF-like protein produced by type-1 astrocytes. Neuron, 1, 485-94.

2) Results

Results are shown in table 1.
(TER shown as % of 0 hour reading, mean ± standard deviation for triplicate Transwells)

Table 1

Compound	Content	TER, % at 1.5h	at 2.5h	at 24h
Rolipram	10μM	257±29	237±36	118±16
Control 1	1×10^4nM	292±4	291±13	188±9
Control 1	1×10^3nM	239±12	232±24	124±6
Control 1	1×10^2nM	190±25	173±8	91±5
Control 1	1×10nM	134±10	111±3	63±3
Control 2	1×10^4nM	253±5	246±15	149±9
Control 2	1×10^3nM	184±32	159±14	80±1
Control 2	1×10^2nM	142±7	123±4	75±11
Control 2	1×10nM	128±7	108±2	62±2
Example 2	1×10^4nM	281±16	304±14	120±10
Example 2	1×10^3nM	260±27	257±15	130±5
Example 2	1×10^2nM	173±23	151±15	99±1
Example 2	1×10nM	123±6	98±2	65±1

(2) *In vitro* study: c-AMP Content (Control=1.00)

1) Materials - The following tested compounds correspond to the examples.

2) Method
Brain endothelial cells were cultured on 96 well plates. After attainment of confluence, culture medium was replaced with 50 µl of fresh medium and the cultures were incubated for a further 2 hours at 37°C. Compounds were diluted in pH equilibrated medium at 37°C to give twice the desired final concentration. Then, 50 µl was added in triplicate to the cultures for 2 hours. In order to extract cellular cAMP, the medium was then rapidly replaced with ice-cold 0.1M-HCl. After 30 minutes at 4°C, the extract was assayed following acetylation by radioimmuno-scintillation proximity assay (Amersham RIA-SPA: RPA 538).

3) Results - Results are shown in table 2.

Table 2

Compound	cyclic AMP content			
	1µM	10µM	100µM	Solubility in water
Control 1				insol.
Control 2	1.32	1.93	5.61	insol.
Example 1	1.92	2.90	4.85	sol.
Example 2	2.63	5.68	6.93	sol.
Example 3	1.26	1.75	3.19	sol.
Example 5	1.33	1.33	1.50	sol.
Example 6	1.04	1.14	1.33	sol.
Example 7	1.03	1.11	0.81	insol.
Example 9	1.37	1.71	2.38	sol.
Example 10	1.27	1.33	1.50	sol.
Example 12	1.07	1.55	1.94	sol.
Example 13	1.39	1.22	1.50	sol.
Example 14	1.02	0.95	1.30	sol.
Example 15	0.86	1.62	1.41	sol.
Example 16	1.08	1.16	1.54	sol.

Most of the present invention compounds are soluble in water, whereas Control 1 and 2 are insoluble. This physical property is very advantageous for the treatment of cerebrovascular diseases, because water soluble materials can be formulated easily and stably as an injection form and can pass through BBB.

(3) *In vivo* study: Effect of the present invention compound on BBB integrity in the middle cerebral artery occlusion model in rats

1) Material
The compound of example 2 was used as a representative of the present invention.

2) Method

Male Sprague-Dawley rats (270-320g) were anesthetized with halothane and subjected to 120 min of temporary MCAo by retrograde insertion of an intraluminal nylon suture[1)] coated with poly-L-lysine[2)] through the external carotid artery into the internal carotid artery and MCA. Temperature probes were inserted in the rectum and the left temporalis muscles. Heating lamps were used to maintain rectal and temporalis muscle temperatures at 37 to 38 °C. In all rats, polyethylene catheters were introduced into the right femoral artery and vein for blood pressure recording, blood sampling, for Evans Blue and drug infusion. Mean arterial blood pressure (hereinafter abbreviated as MABP), plasma glucose and blood gases were continuously measured during the operation.

The neurological status was evaluated during occlusion (60 min) in all groups; 3 and 5 hours after MCAo in A and B groups; 24 and 48 hours in C and D groups. A grading scale of 0-12 was used to assess the effects of occlusion (normal score -0; maximal score -12; Table 3).

1) ; Zea Longa, E.L., Einstein, P.R., Carlson, S. and Cummins, R., Reversible middle cerebral artery occlusion without craniectomy in rats, Stroke, 20 (1989) 84-91.

2) ; Belayev, L., Alonso, O.F., Busto, R., Zhao, W. and Ginsberg, M.D., Middle cerebral artery occlusion in the rat by intraluminal suture: neurological and pathological evaluation of an improved model, Stroke, 27 (1996) 1616-1623.

Table 3 Neurological evaluation of rats with MCAo

Item	Normal Score	Deficit
Postual Reflex ("Hang Test")*	0	2
Placing Test** (performed on each side)		
Visual Placing		
Forward	0	2
Sideways	0	2
Tactile Placing		
Dorsal Surface of Paw	0	2
Lateral Surface of Paw	0	2
Proprioceptive Placing	0	2
Total Score	0	12

* Postural Reflex ("Hang Test")	** Placing Test
0-no observable deficit	0-complete immediate placing
1-limb flexion during hang test	1-incomplete and / or delayed placing (<2sec.)
2-deficit on lateral push	2-absence of placing

MCAo, middle cerebral artery occlusion

3) Drug infusion

Tested compound (example 2 in saline, 1 mg/kg, i.v.) or vehicle (0.9% saline) was administered by infusion after the onset of MCAo (Table 4).

Table 4 Experimental Groups

Group	n	Procedure	MCAo (min)	Evans Blue injection	Sacrificed	Treatment	Dose	Route
A (Vehicle) Saline-5h	5	MCAo	120	3h	5h	0-5h	2mg/Kg	i.v.
B (Example 2) 1mg/Kg-5h	5	MCAo	120	3h	5h	0-5h	1mg/Kg	i.v.
C (Vehicle) Saline-48h	9	MCAo	120	46h	48h	0-48h	1mg/Kg	i.v.
D (Example 2) 1mg/Kg-48h	8	MCAo	120	46h	48h	0-48h	1mg/Kg	i.v.

Four animal groups were studied: Groups A and B were treated by infusion of vehicle or drug over 5 hours, and Groups C and D were treated by infusion of vehicle or drug over 48 hours.

4) Evaluation of BBB integrity

The integrity of the BBB was investigated using Evans-Blue extravasation, according to Uyama et al.[3]. Animals were divided into groups as listed in Table 4. Evans Blue (EB, 2%, in saline, 4 ml/Kg) was injected intravenously at 3 h after the onset of MCAo in Groups A and B; and at 46 h in Groups C and D. The chest was subsequently opened under halothane anesthesia 2 h later. Rats were perfused with saline through the left ventricle at 110 mm Hg pressure until colorless perfusion fluid was obtained from the right atrium.

After decapitation, the brain was blocked into 2 segments that included the levels bregma +2.7 and -0.3 mm. Coronal blocks were next divided into right and left hemispheres and were cut into six regions for local measurement of EB dye (Fig. 1). Samples were weighed and placed in 50% trichloroacetic acid solution. Following homogenization and centrifugation, the extracted dye was diluted with ethanol (1:3), and its fluorescence was determined (excitation at 620nm and emission at 680nm) with a Perkin-Elmer LS-5B Luminescence spectrometer.

Calculations were based on external standards in the same solvent (100-500 ng/ml). The tissue content of EB was quantified from a linear standard curve derived from known amounts of the dye and was expressed per gram of tissue.

3) ; Uyama, O., Okamura, N., Yanase, M., Narita, M., Kawabata, K. and Sugita, M., Quantitative evaluation of vascular permeability in the gerbil brain after transient ischemia using Evan's blue fluorescence, J Cereb. Blood Flow Metab., 8 (1988) 282-284.

5) Results

Rectal and cranial (temporalis muscle) temperatures, MABP, blood gases and plasma glucose in the 27 animals of this study showed no significant differences between groups (Tables 5-9).

Table 10 summarises the neurological outcome after MCAo. The neurological scores at 3 h and 5 h after MCAo were significantly better in the compound 2 treated group (1 mg/kg, 5-hour infusion) than in the vehicle-treated group (mean ± SE; 6.2 ± 0.5 vs. 8.4 ± 0.2; 5.8 ± 0.6 vs. 8.4 ± 0.2, respectively; A vs B, $p < 0.003$).

The compound 2 also significantly improved the neurological score at 60 min, 24 h and 48 h in the compound 2 treated group (1 mg/kg. 48-hours infusion) compared to the vehicle group (mean ± SE; 7.3 ± 0.5 vs. 9.0 ± 0; 4.7 ± 0.4 vs. 6.7 ± 0.4; 4.5 ± 0.3 vs. 7.0 ± 0.4 respectively; C vs D. $p < 0.004$).

The effect of the compound 2 on BBB integrity after MCAo is shown in Table 11. The compound 2 (1 mg/kg, 5 hours infusion) significantly decreased in the dye extravasation (Fig. 2) into the cortex (mean ± SE; 9.3 ± 2.9 vs. 24.3 ± 5.8 μg/g. sample 3, $p=0.05$), striatum (26.4 ± 3.1 vs. 47.3 ± 4.7 μg/g, sample 5. $p= 0.01$) and right hemisphere (41.2 ± 5.4 vs. 82.4 ± 9.2 μg/g, $p= 0.005$) compared to the vehicle-treated group. Total EB from whole brain was also significantly decreased in the compound 2 treated rats compared with the vehicle group (63.9 ± 10.5 vs. 111.8 ± 12.9 μg/g; $p=0.02$).

The compound 2 (1 mg/Kg, 48 hours infusion) also significantly decreased dye extravasation (Fig. 3) into the cortex (mean ± SE; 7.4 ± 2.5 vs. 29.0 ± 8.3 μg/g, sample 3, $p= 0.05$). striatum (17.2 ± 2.2 vs. 50.8 ± 12.1 μg/g. sample 5, $p= 0.03$) and right hemisphere (30.7 ± 4.0 vs. 93.2 ± 18 μg/g, $p=0.01$) compared to the vehicle-treated group. Total EB from whole brain was also significantly decreased in the compound 2 treated rats compared with the vehicle group (44.8 ± 6,2 vs. 118.9 ± 19.6 μg/g; $p=0.01$).

Four animals died in our study (two in group C and two in group D). None died in Groups A or B (vehicle and the compound 2 treated rats).

Table 5 Head Temperature Changes in Rats

Groups	No. of animal (min)	Before MCAo (min)	During MCAo (h)			After MCAo				
		15	0	15	120	2.15	3	5	24	48
A (Vehicle) Saline-5h	6 10303	37.2	37.1	37.1	37.0	37.1	37.2	37.1		
	6 11042	37.3	37.2	37.1	37.3	37.2	37.4	37.2		
	6 11074	37.2	37.2	37.1	37.1	37.2	37.2	37.3		
	7 01313	37.0	37.0	37.1	37.0	37.1	37.0	37.0		
	7 02061	37.2	37.1	37.2	37.3	37.1	37.0	37.2		
Average		37.2	37.1	37.1	37.1	37.1	37.2	37.2		
S.D.		0.11	0.08	0.04	0.15	0.05	0.17	0.11		
S.E.		0.05	0.04	0.02	0.07	0.02	0.07	0.05		
B (Example2) 1mg/Kg-5h	6 11261	37.2	37.2	37.1	37.3	37.4	37.1	37.0		
	7 01092	37.3	37.2	37.0	37.2	37.1	37.0	37.0		
	7 01103	37.0	37.0	37.1	37.1	37.1	37.1	37.2		
	7 01173	37.1	37.0	37.0	37.0	37.0	37.1	37.2		
	7 01232	37.2	37.1	37.2	37.0	37.0	37.1	37.0		
Average		37.2	37.1	37.1	37.1	37.1	37.1	37.1		
S.D.		0.11	0.10	0.08	0.13	0.16	0.04	0.11		
S.E.		0.05	0.04	0.04	0.06	0.07	0.02	0.05		
C (Vehicle) Saline-48	6 11063	37.2	37.1	37.0	37.2	37.1			37.3	37.0
	6 11111	37.3	37.2	37.1	37.1	37.2			37.1	37.2
	7 01283	37.2	37.4	37.3	37.0	37.0			37.4	37.0
	7 01284	37.2	37.1	37.0	37.0	37.1			37.9	37.9
	7 02031	37.3	37.2	37.1	37.0	37.1			died	
	7 02042	37.0	37.0	37.0	37.0	37.1			37.0	37.1
	7 02043	37.0	37.1	37.0	37.1	37.0			died	
	7 02052	37.2	37.1	37.2	37.3	37.1			37.2	37.0
	7 02053	37.1	37.1	37.0	37.1	37.2			37.1	37.5
Average		37.2	37.1	37.1	37.1	37.1			37.3	37.2
S.D.		0.11	0.11	0.11	0.11	0.07			0.3	0.3
S.E.		0.04	0.04	0.04	0.04	0.02			0.1	0.1
D (Example 2) 1mg/Kg-48	6 11054	37.3	37.1	37.2	37.4	37.3			37.3	37.1
	6 11123	37.2	37.1	37.2	37.3	37.3			37.0	37.8
	7 11124	37.0	37.1	37.2	37.1	37.0			died	
	6 11253	37.3	37.2	37.1	37.2	37.3			36.3	died
	7 01143	37.3	37.2	37.1	37.0	37.0			37.0	37.0
	7 01215	37.1	37.1	37.1	37.2	37.1			38.3	37.0
	7 01272	37.3	37.2	37.2	37.0	37.0			36.4	37.0
	7 01273	37.2	37.2	37.2	37.0	37.0			37.3	37.0
Average		37.2	37.2	37.2	37.2	37.1			37.1	37.2
S.D.		0.11	0.05	0.05	0.15	0.15			0.7	0.3
S.E.		0.04	0.02	0.02	0.05	0.05			0.3	0.1
T-test(Avs.B)	0.78	0.74	0.37	0.83	0.80					
T-test(Cvs.D)		0.41	0.90	0.06	0.34	0.66			0.48	0.63

Table 6 Rectal Temperature Changes in Rats

Groups	No. of animal	Before MCAo (min)	During MCAo (min)				After MCAo (h)				
		15	0	15	60	120	2.15	3	5	24	48
A (Vehicle) Saline-5h	6 10303	37.4	37.3	37.4	37.3	37.2	37.3	37.4	37.4		
	6 11042	37.5	37.4	37.3	37.4	37.5	37.4	37.5	37.4		
	6 11074	37.5	37.4	37.3	37.4	37.3	37.4	37.3	37.5		
	7 01313	37.2	37.1	37.3	37.3	37.2	37.3	37.0	37.2		
	7 02061	37.4	37.3	37.3	37.4	37.5	37.2	37.3	37.4		
Average		37.4	37.3	37.3	37.4	37.3	37.3	37.3	37.4		
S.D.		0.12	0.12	0.04	0.05	0.15	0.08	0.19	0.11		
S.E.		0.05	0.05	0.02	0.02	0.07	0.04	0.08	0.05		
B (Example2) 1mg/Kg-5h	6 11261	37.5	37.4	37.3	37.4	37.5	37.5	37.3	37.2		
	7 01092	37.5	37.4	37.1	37.3	37.4	37.3	37.1	37.1		
	7 01103	37.2	37.1	37.3	37.4	37.3	37.2	37.1	37.4		
	7 01173	37.3	37.1	37.2	37.2	37.1	37.2	37.3	37.4		
	7 01232	37.4	37.3	37.4	37.3	37.1	37.2	37.3	37.0		
Average		37.4	37.3	37.3	37.3	37.3	37.3	37.2	37.2		
S.D.		0.13	0.15	0.11	0.08	0.18	0.13	0.11	0.18		
S.E.		0.06	0.07	0.05	0.04	0.08	0.06	0.05	0.08		
C (Vehicle) Saline-48h	6 11063	37.4	37.3	37.2	37.1	37.4	37.3			37.5	37.2
	6 11111	37.5	37.4	37.3	37.4	37.3	37.4			37.4	37.5
	7 01283	37.4	37.5	37.5	37.3	37.1	37.2			37.7	37.2
	7 01284	37.4	37.3	37.2	37.1	37.2	37.3			38.2	38.4
	7 02031	37.5	37.4	37.3	37.2	37.2	37.3			died	
	7 02042	37.1	37.2	37.3	37.0	37.2	37.3			37.2	37.3
	7 02043	37.2	37.3	37.1	37.0	37.2	37.1			died	
	7 02052	37.4	37.3	37.4	37.3	37.5	37.4			37.5	37.0
	7 02053	37.4	37.3	37.2	37.4	37.3	37.4			37.3	38.0
Average		37.4	37.3	37.3	37.2	37.3	37.3			37.5	37.5
S.D.		0.13	0.09	0.12	0.16	0.12	0.10			0.33	0.50
S.E.		0.04	0.03	0.04	0.05	0.04	0.03			0.13	0.19
D (Example 2) 1mg/Kg-48h	6 11054	37.5	37.3	37.4	37.3	37.6	37.5			37.5	37.3
	6 11123	37.4	37.3	37.3	37.5	37.4	37.5			37.1	38.1
	7 11124	37.2	37.3	37.4	37.4	37.3	37.2			died	
	6 11253	37.5	37.4	37.3	37.5	37.4	37.5			36.5	died
	7 01143	37.5	37.4	37.3	37.3	37.0	37.1			37.2	37.0
	7 01215	37.3	37.2	37.3	37.2	37.4	37.3			38.6	37.0
	7 01272	37.5	37.4	37.4	37.3	37.1	37.1			36.7	37.0
	7 01273	37.4	37.3	37.4	37.2	37.0	37.2			37.6	37.2
Average		37.4	37.3	37.4	37.3	37.3	37.3			37.3	37.3
S.D.		0.11	0.07	0.05	0.12	0.22	0.18			0.69	0.43
S.E.		0.04	0.03	0.02	0.04	0.08	0.06			0.26	0.17
T-test(Avs.B)	0.81	0.66	0.31	0.40	0.58	0.58					
T-test(Cvs.D)	0.46	0.83	0.14	0.06	0.92	1.00			0.45	0.37	

Table 7 Arterial Blood Pressure in Rats

Groups	No. of animal	15min Before MCAo	MCAo Occlusion (min) 0	15	120	After MCAo 2.15h
A (Vehicle) Saline-5h	6 10303	110	110	120	100	90
	6 11042	120	130	115	105	105
	6 11074	80	90	110	70	70
	7 01313	105	125	110	80	85
	7 02061	100	130	110	90	80
Average		103.0	117.0	113.0	89.0	86.0
S.D.		14.8	17.2	4.5	14.3	12.9
S.E.		6.6	7.7	2.0	6.4	5.8
B (Example2) 1mg/Kg-5h	6 11261	130	120	125	110	105
	7 01092	115	120	120	90	70
	7 01103	130	130	120	105	105
	7 01173	100	110	120	80	80
	7 01232	80	110	110	80	90
Average		111.0	118.0	119.0	93.0	90.0
S.D.		21.3	8.4	5.5	14.0	15.4
S.E.		9.5	3.7	2.4	6.2	6.9
C (Vehicle) Saline-48h	6 11063	90	110	110	110	90
	6 11111	110	130	130	120	110
	7 01283	90	120	110	100	90
	7 01284	100	130	105	80	95
	7 02031	90	100	85	80	90
	7 02042	90	120	120	90	90
	7 02043	90	90	90	80	80
	7 02052	85	90	105	80	80
	7 02053	85	120	110	110	85
Average		92.2	112.2	107.2	94.4	90.0
S.D.		7.9	15.6	13.7	15.9	9.0
S.E.		2.6	5.2	4.6	5.3	3.0
D (Example 2) 1mg/Kg-48h	6 11054	120	120	110	100	90
	6 11123	80	85	90	90	80
	7 11124	95	100	110	70	70
	6 11253	100	110	80	80	75
	7 01143	110	130	140	90	90
	7 01215	90	110	100	90	80
	7 01272	90	100	110	80	80
	7 01273	80	110	100	80	90
Average		95.6	108.1	105.0	85.0	81.9
S.D.		14.0	13.6	17.7	9.3	7.5
S.E.		4.9	4.8	6.3	3.3	2.7
T-test(Avs.B)		0.51	0.91	0.09	0.67	0.67
T-test(Cvs.D)		0.54	0.58	0.78	0.16	0.06

Table 8 Arterial Blood Gates in Rats

Groups	No. of animal	15min Before MCAo			During MCAo (15min)		
		pH	pO_2	pCO_2	pH	pO_2	pCO_2
A (Vehicle) Saline-5h	6 10303	7.41	95.6	38.4	7.42	105.3	38.3
	6 11042	7.41	108.5	46.6	7.43	101.6	47.5
	6 11074	7.42	92.5	38.7	7.40	98.6	40.5
	7 01313	7.30	116.7		7.30	116.6	
	7 02061	7.46	98.9	37.2	7.43	82.7	40.4
Average		7.40	102.4	40.2	7.40	101.0	41.7
S.D.		0.06	10.0	4.3	0.06	12.3	4.0
S.E.		0.03	4.5	2.1	0.02	5.5	2.0
B (Example2) 1mg/Kg-5h	6 11261	7.42	128.9	38.9	7.41	118.1	38.3
	7 01092	7.43	94.7	40.3	7.40	106.3	39.0
	7 01103	7.37	109.7	39.9	7.40	99.3	37.5
	7 01173	7.40	124.4	41.2	7.43	125.8	38.9
	7 01232	7.43	101.2	36.2	7.42	108.8	40.3
Average		7.41	111.8	39.3	7.41	111.7	38.8
S.D.		0.03	14.7	1.9	0.01	10.4	1.0
S.E.		0.01	6.6	0.9	0.01	4.6	0.5
C (Vehicle) Saline-48h	6 11063	7.37	120.6	40.5	7.39	92.4	37.1
	6 11111	7.41	91.9	41.5	7.41	96.4	40.4
	7 01283	7.43	113.0	37.4	7.39	107.5	40.6
	7 01284	7.41	96.6	39.5	7.41	99.3	34.8
	7 02031	7.33	98.4	38.2	7.34	101.4	38.6
	7 02042	7.42	123.3	37.6	7.38	108.4	39.4
	7 02043	7.38	105.7	40.3	7.34	96.1	38
	7 02052	7.38	100.1	37.8	7.39	96.5	38.4
	7 02053	7.42	98.7	38.2	7.43	85.3	41.9
Average		7.39	105.4	39.0	7.39	98.1	38.8
S.D.		0.03	11.1	1.5	0.03	7.2	2.1
S.E.		0.01	3.7	0.5	0.01	2.4	0.7
D (Example 2) 1mg/Kg-48h	6 11054	7.4	94.0	33.6	7.38	91.6	42.2
	6 11123	7.43	99.2	34.0	7.37	97.4	39.2
	7 11124	7.41	98.0	37.1	7.36	98.6	40.7
	6 11253	7.39	94.7	38.9	7.42	105	38.4
	7 01143	7.42	96.6	38.6	7.40	100.7	40.6
	7 01215	7.52	95.9	38.6	7.35	86.8	41.4
	7 01272	7.42	106.1	40.3	7.42	105.4	36
	7 01273	7.39	101.7	42.2	7.39	101.2	39.7
Average		7.42	98.28	37.91	7.39	98.34	39.78
S.D.		0.04	4.02	2.94	0.03	6.41	1.95
S.E.		0.01	1.42	1.04	0.01	2.27	0.69
T-test (A vs. B)		0.84	0.22	0.43	0.66	0.17	0.17
T-test (C vs. D)		0.10	0.08	0.05	0.67	0.70	0.12

Table 9 Arterial Glucose in Rats

Groups	No. of animal	15min Before MCAo	During MCAo (15min)
A (Vehicle) Saline-5h	6 10303	95	84
	6 11042	122	105
	6 11074	131	137
	7 01313	98	112
	7 02061	146	111
Average		118.4	109.7
S.D.		21.8	19.1
S.E.		9.8	8.5
B (Example2) 1mg/Kg-5h	6 11261	130	115
	7 01092	139	168
	7 01103	171	138
	7 01173	111	141
	7 01232	130	144
Average		136.2	141.2
S.D.		22.0	18.9
S.E.		9.8	8.4
C (Vehicle) Saline-48h	6 11063	121	118
	6 11111	138	112
	7 01283	103	100
	7 01284	108	114
	7 02031	125	144
	7 02042	104	110
	7 02043	106	113
	7 02052	94	110
	7 02053	96	114
Average		110.6	115.0
S.D.		14.5	11.9
S.E.		4.8	4.0
D (Example 2) 1mg/Kg-48h	6 11054	133	101
	6 11123	99	114
	7 11124	96	117
	6 11253	121	111
	7 01143	200	139
	7 01215	108	134
	7 01272	120	129
	7 01273	154	128
Average		128.9	121.6
S.D.		34.3	12.9
S.E.		12.1	4.6
T-test (A vs. B)		0.23	0.09
T-test (C vs. D)		0.16	0.29

Table 10 Neurological Outcome Following 120min MCAo in Rats

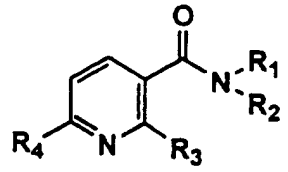
Groups	No. of animal	MCAo (min)	Score before MCAo 15min	Score during MCAo 60min	Score After MCAo 3h	5h	24h	48h
A (Vehicle) Saline-5h	6 10303	120	0	9	8	8		
	6 11042	120	0	8	8	8		
	6 11074	120	0	9	9	9		
	7 01313	120	0	9	9	9		
	7 02061	120	0	8	8	8		
Average				8.6	8.4	8.4		
S.D.				0.5	0.5	0.5		
S.E.				0.2	0.2	0.2		
B (Example2) 1mg/Kg-5h	6 11261	120	0	6	6	5		
	7 01092	120	0	8	6	6		
	7 01103	120	0	8	8	8		
	7 01173	120	0	6	6	5		
	7 01232	120	0	9	5	5		
Average				7.4	6.2	5.8		
S.D.				1.3	1.1	1.3		
S.E.				0.6	0.5	0.6		
C (Vehicle) Saline-48h	6 11063	120	0	9			8	9
	6 11111	120	0	9			7	7
	7 01283	120	0	9			5	6
	7 01284	120	0	9			7	7
	7 02031	120	0	9			died	
	7 02042	120	0	9			6	6
	7 02043	120	0	9			died	
	7 02052	120	0	9			8	8
	7 02053	120	0	9			6	6
Average				9			6.7	7.0
S.D.				0			1.1	1.2
S.E.				0			0.4	0.4
D (Example 2) 1mg/Kg-48h	6 11054	120	0	9			5	6
	6 11123	120	0	6			4	4
	7 11124	120	0	5			died	
	6 11253	120	0	8			6	died
	7 01143	120	0	8			6	5
	7 01215	120	0	8			4	4
	7 01272	120	0	8			4	4
	7 01273	120	0	6			4	4
Average				7.3			4.7	4.5
S.D.				1.4			1.0	0.8
S.E.				0.5			0.4	0.3
T-test(Avs.B)				0.10	0.004	0.003		
T-test(Cvs.D)				0.002			0.004	0.001

Table 11 Tissue Evans Blue Content

Groups	No. of animal	Evans Blue (μg/g tissue)				Hemispheres EB (μg/g tissue)				Total EB μg/g
		Samp. 1	Samp. 2	Samp. 3	Samp. 4	Samp. 5	Samp. 6	right	left	
A (Vehicle) Saline-5h	6 10303	11.13	7.87	25.48	10.49	32.41	13.81	69.0	32.2	101.2
	6 11042	9.62	7.99	16.81	9.76	50.28	15.55	76.7	31.3	108.0
	6 11074	18.69	10.57	48.28	15.74	52.85	16.27	117.8	42.6	160.4
	7 01313	7.59	5.53	18.03	6.24	41.61	4.26	67.2	16.0	83.3
	7 02061	7.18	9.11	14.82	7.52	59.27	8.1	81.3	24.7	106.0
Average		10.8	8.2	24.3	10.0	47.3	11.2	82.4	29.4	111.8
S.D.		4.7	1.9	12.9	3.7	10.5	4.9	20.6	9.8	28.9
S.E.		2.09	0.83	5.79	1.64	4.67	2.19	9.21	4.39	12.92
B (Example2) 1mg/Kg-5h	6 11261	8.03	9.68	17.3	13.5	37.16	16.38	62.5	39.6	102.1
	7 01092	1.76	2.28	12.07	5.77	22.13	14.11	36.0	22.2	58.1
	7 01103	6.89	7.94	11.8	9.44	19.03	13.53	37.7	30.9	68.6
	7 01173	6.32	2.52	1.42	8.36	28.9	0.7	36.6	11.6	48.2
	7 01232	4.02	2.4	4.07	3.47	24.91	3.76	33.0	9.6	42.6
Average		5.4	5.0	9.3	8.1	26.4	9.7	41.2	22.8	63.9
S.D.		2.5	3.6	6.5	3.8	7.0	7.0	12.1	12.7	23.5
S.E.		1.12	1.59	2.89	1.70	3.14	3.12	5.39	5.69	10.51
C (Vehicle) Saline-48h	6 11063	20.54	12.67	13.12	9.4	24.85	10.27	58.5	32.3	90.9
	6 11111	15.42	7.24	14.48	6.38	21.43	5.82	51.3	19.4	70.8
	7 01283	6.08	5.02	65.85	9.03	81.97	8.16	153.9	22.2	176.1
	7 01284	14.59	13.11	54.38	8.9	68.97	12.91	137.9	34.9	172.9
	7 02042	11.9	7.2	12.32	5.8	35.61	5.47	59.8	18.5	78.3
	7 02052	10.89	8.22	17.23	6.03	23.49	5.14	51.6	19.4	71.0
	7 02053	14.52	7.41	25.69	11.86	99.04	13.72	139.3	33.0	172.2
Average		13.4	8.7	29.0	8.2	50.8	8.8	93.2	25.7	118.9
S.D.		4.5	3.0	22.0	2.2	32.0	3.6	47.6	7.4	51.8
S.E.		1.69	1.14	8.30	0.84	12.09	1.35	18.00	2.78	19.56
D (Example 2) 1mg/Kg-48h	6 11054	9.42	8.55	9.73	9.64	15.81	12.12	35.0	30.3	65.3
	6 11123	8.04	5.07	18.91	5.12	14.97	0.23	41.9	10.4	52.3
	7 01143	1.99	1.23	4.43	0.83	15.36	3.99	21.8	6.1	27.8
	7 01215	3.04	2.21	2.25	2.63	20.68	3.71	26.0	8.6	34.5
	7 01272	8.76	6.2	5.67	5.55	26.01	3.93	40.4	15.7	56.1
	7 01273	5.24	6.49	3.22	2.74	10.43	4.33	18.9	13.6	32.5
Average		6.1	5.0	7.4	4.4	17.2	4.7	30.7	14.1	44.8
S.D.		3.1	2.8	6.2	3.1	5.4	3.9	9.8	8.7	15.2
S.E.		1.28	1.13	2.54	1.26	2.20	1.60	4.00	3.53	6.19
T-test(Avs.B)		0.05	0.11	0.05	0.46	0.01	0.70	0.005	0.39	0.02
T-test(Cvs.D)		0.01	0.04	0.04	0.03	0.03	0.08	0.01	0.02	0.01

CLAIMS

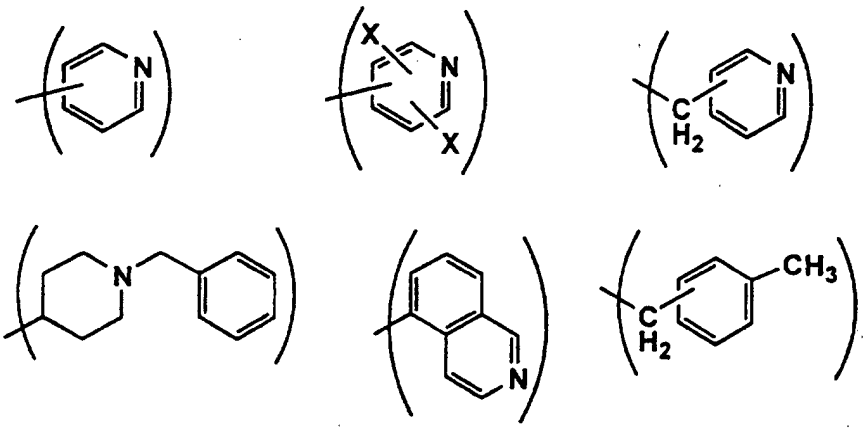
1. A compound of general formula (I) or a pharmacologically acceptable salt thereof:



(I)

wherein R^1 represents a hydrogen atom or a lower alkyl group;

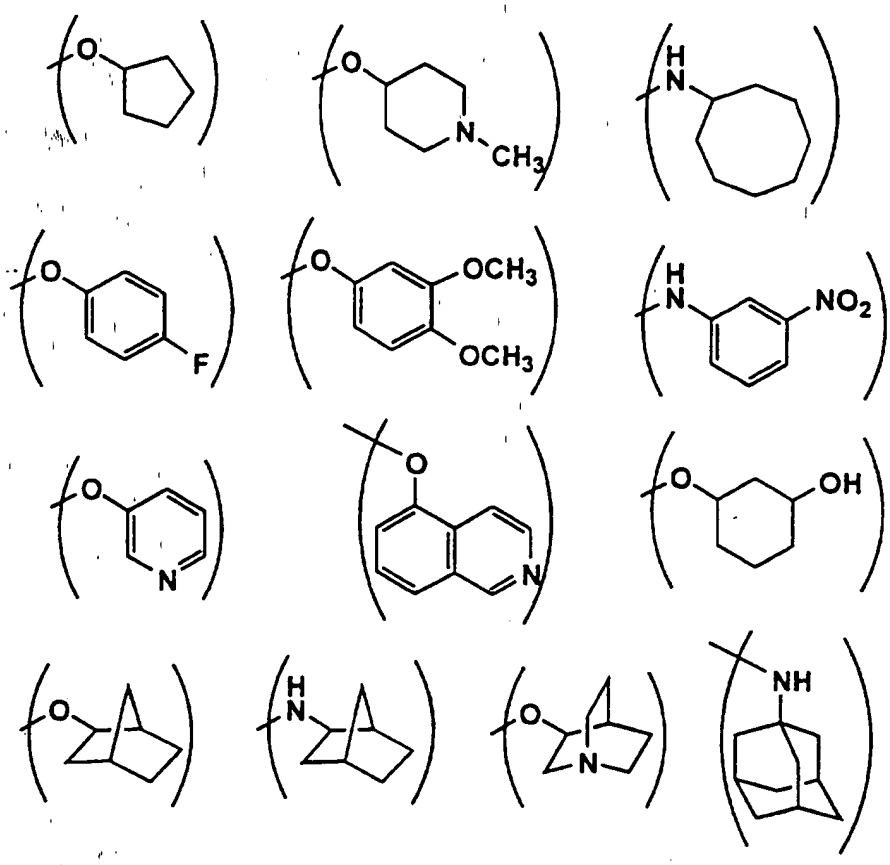
R^2 represents a group selected from the following:



wherein X represents a halogen atom;

or R^1 and R^2 can form a 4-methylpiperazinyl group together which may be substituted;

R^3 represents a group selected from the following group;



R^4 represents a hydrogen atom or a lower alkoxy group.

2. A compound or a pharmacologically acceptable salt thereof as claimed in claim 1, which is a compound selected from:
(1) N-(4-pyridyl)-2-cyclopentyloxynicotinicamide,
(2) N-(4-pyridyl)-2-exonorbornyloxynicotinic amide,
(3) N-(4-pyridyl)-2-(4-fluorophenyloxy) nicotinic amide,
(4) N-(4-pyridyl)-2-(3-hydroxycyclohexyloxy) nicotinic amide,
(5) N-(4-pyridyl)-2-(2-exonorbornylamino) nicotinic amide,
(6) N-(4-pyridyl)-2-cyclooctylaminonicotinic amide,
(7) N-(4-pyridyl)-2-adamantylaminonicotinic amide,
(8) N-(4-pyridyl)-2-exonorbornyloxy-6-methyl nicotinic amide,
(9) N-(3-pyridyl)-2-exonorbornyloxynicotinic amide,
(10) N-(3,5-dichloro-4-pyridyl)-2-(2-exonorbornyloxy) nicotinic amide,
(11) N-(5-isoquinolynyl)-2-(2-exonorbornyloxy) nicotinic amide,
(12) N-(4-picolyl)-2-(4-fluorophenyloxy) nicotinic amide,
(13) N-(4-picolyl)-2-(3-nitrophenylamino) nicotinic amide,
(14) N-(3-picolyl)-2-(2-Exonorbornyloxy) nicotinic amide,
(15) N-(3-picolyl)-2-(3,4-dimethoxyphenyloxy) nicotinic amide and;

(16) N-(1-Benzyl-4-piperidyl)-2-(2-exonorbornyloxy) nicotinic amide.

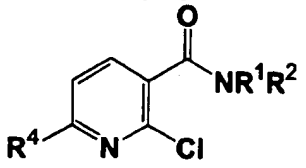
3. A pharmaceutical composition comprising a therapeutically or ameliorative effective amount of a compound or a pharmacologically acceptable salt thereof as defined in claim 1 and a pharmacologically acceptable vehicle.

4. The use of a compound or a pharmacologically acceptable salt thereof as defined in claim 1 for the making of a medicament for treating or ameliorating a disease against which phosphodiesterase antagonism is efficacious.

5. A method for treating or ameliorating a disease which comprises administering a pharmaceutically effective amount of a compound or a pharmacologically acceptable salt thereof as defined in claim 1 to a patient suffering from a disease against which phosphodiesterase antagonism is efficacious.

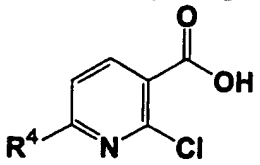
6. A method for treating or ameliorating a disease which comprises administering a pharmaceutically effective amount of a compound or a pharmacologically acceptable salt thereof as defined in claim 1 to a patient suffering from a variety of allergic and inflammatory diseases such as asthma, chronic bronchitis, shock, rheumatoid arthritis, stroke, reperfusion injury, encephalomyelitis and multiple sclerosis.

7. A process for preparation of a compound of general formula (II),



(II)

comprising derivatising an optionally protected compound of general formula (III),



(III)

and optionally thereafter converting the compound of general formula (II) so formed into another compound of general formula (II), in which R^1 to R^4 have the same meaning as defined in claim 1.

8. A process as claimed in claim 7, in which the derivativisation of the compound of general formula (III) to form a compound of general formula (II) is treatment with:

(a) a chlorinating agent

(b) a mixed acid anhydride forming agent or,

(c) 1,3-dicyclohexylcarbodiimide (DCC),
and a primary or secondary amine of general formula (IV),

HNR^1R^2 (IV)

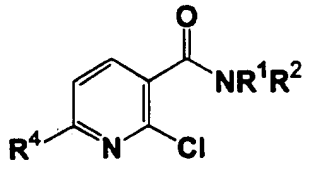
(wherein R^1 and R^2 have the same meaning as defined in claim 1).

9. A process as claimed in claim 8, in which the chlorinating agent is thionyl chloride, sulfuryl chloride, oxalyl chloride, phosphorus pentachloride, phosphorous trichloride or phosphorous oxychloride.

10. A process as claimed in claim 8, in which the mixed acid anhydride forming agent is methyl chloroformate or ethyl chloroformate.

11. A process as claimed in any one of claims 8 to 10, in which the compound of general formula (II) is further treated with an alcohol or an amine to give a compound of general formula (I).

12. A compound of general formula (II), in which R^1 to R^4 have the same meaning as defined in claim 1,



(II)

with the proviso that N-(4-pyridyl)-2-chloronicotinic amide, N-(3-pyridyl)-2-chloronicotinic amide and N-(2-pyridyl)-2-chloronicotinic amide are excluded.

13. A compound as claimed in claim 12 which is,
(1) N-(4-pyridyl)-2-chloro-6-methyl nicotinic amide,
(2) N-(3,5-dichloro-4-pyridyl)-2-chloronicotinic amide,
(3) N-(5-isoquinolyl)-2-chloronicotinic amide,
(4) N-(4-picolyl)-2-chloronicotinic amide,
(5) N-(3-picolyl)-2-chloronicotinic amide and;
(6) N-(N-benzylpiperidin-4-yl)-2-chloronicotinic amide.

The Patent Office

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Application No: GB 9810659.4 **Examiner:** Anwar Gilani
Claims searched: 1-6 **Date of search:** 9 September 1998

Patents Act 1977
Amended Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): C2C (CKH, CKJ, CKP, CKT)

Int Cl (Ed.6): C07D 213/82, 401/12, 401/14

Other: Online: CAS-ONLINE, WPI, EDOC

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	EP0773024 A2	(PFIZER) p.5 l.15-20, claim 1	1,3,4
X	US4861891	(SACCOMANO ET AL) compound 7 in the table of cols.13/14 and claim 1	1,3,4
A	JP50082075	(HISAMITSU PHARMACEUTICAL) see examples	1,3,6 at least
X	Chem. Abs. 111:153648 & JP01113369 A2 (MITSUBISHI PETROCHEMICAL CO.) see abstract		1,2
A	Chem. Abs. 120:217217 & Khim.-Farm. Zh. (1993), 27(7), 34-5 L.M.Demina et al, "Alkylamides of 2-chloro- and 2-arylamino-4,6-dimethylnicotinic acid ..." see abstract		1

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.